

## AMENDMENTS

Please amend the application as indicated hereafter.

### In the Specification

Please substitute the following clean-copy paragraph/page text for the pending page/paragraph text of the same number.

Page 40, last paragraph, line 21 – page 43, first paragraph, line 5:

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From a single specimen of sea anemone *Actinia equina* L., total RNA was isolated by disrupting the tissue in a liquid nitrogen. Two grams of ground, deeply frozen tissue was transferred into 20 ml of guanidinium thiocyanate solution (5.5 M GTC, 0.5 M EDTA, 0.2 M  $\beta$ -mercapto-ethanol) and homogenized in an electrical mixer (16,000 rpm, 4x 30 sec.). Subsequently, the solution was centrifuged at 6000 X g, 20 min. at 15°C. 10 ml of clear supernatant was transferred to 10 ml of Cesium TFA solution ( $\rho$ =1.5 mg/ml), supplemented with 2.5 ml of 0.5 M EDTA, pH=8.0. Sample was centrifuged for 24h at 125,000 X g, 15°C. The supernatant was removed and total RNA (pellet) was dissolved in 1 ml of proteinase K solution (0.5 mg/ml). After incubating the solution at 50°C, 1/2h, total RNA was precipitated by 1/10 volume of 3M KOAc, pH=5.2 and 2.5 vol. of 96% ethanol and the mixture was placed overnight in the freezer at -20°C. Total RNA was pelleted at 8000xg and the pellet was dissolved in 2 ml of TE buffer. 1 ml of dissolved sample was heated at 65°C for 5 min, cooled on ice and subsequently, 0.2 ml of TE buffer, supplemented with 3M NaCl was added. Whole sample was applied to the top of the oligo (dT) – cellulose bed in the column. The cellulose was washed three times with the TE buffer, supplemented with 0.5 M NaCl and 0.1 M NaCl, respectively. Poly(A)+ RNA was eluted with 1 ml (divided into 4 aliquots of 0.25 ml) of TE buffer, prewarmed to 65°C. One third of the sample (approx. 1  $\mu$ g) was used for a synthesis of cDNA according to manufacturer's procedure (Amersham). First strand cDNA synthesis was performed using 11  $\mu$ l of mRNA solution, 4  $\mu$ l 5 X reaction buffer, 1  $\mu$ l Na-pyrophosphate solution, 1  $\mu$ l human placental ribonuclease inhibitor (5U/ $\mu$ l), 2  $\mu$ l of dNTP mix solution and oligo dT primer solution (1  $\mu$ l). After addition of 2  $\mu$ l (10 U/ $\mu$ l) of reverse transcriptase, a mixture was incubated at 42°C for 40 minutes. Second strand cDNA was synthesized by adding the following components: 37.5  $\mu$ l of second strand reaction buffer, 7  $\mu$ l of E. coli DNA polymerase I (4U/ $\mu$ l) and 1  $\mu$ l of E.

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cont A

coli ribonuclease H (1U/μl). The reaction mix was incubated sequentially at 12°C for 60 minutes and 22°C for 60 minutes. After heat denaturation (5 minutes at 70°C), 0.5 μl of T4 DNA polymerase (2U/μl) was added and the reaction mix was incubated at 37°C for 10 minutes. 2.5 μl (250 pmoles) of *Eco*RI-adaptors were ligated to 1 μg of cDNA, using 2 μl of T4 DNA ligase (4U/μl) in 20 μl of ligation mixture. After 8h at 16°C, the ligation mixture was subjected to column purification/size fractionation of adaptor-linked cDNA, using spun columns and TE buffer. Collected fractions of purified cDNA were phosphorylated by T4 polynucleotide kinase (8U/μl) and cDNA was ligated into dephosphorylated λgt11 bacteriophage arms using T4 DNA ligase (40U/μg DNA). Finally, whole ligation mixture was *in vitro* packaged using packaging extract from the same manufacturer (Amersham). A portion of λgt11 cDNA library (10<sup>5</sup> pfu) was used for the infection of Y1090 *E. coli* cells and mixture was plated onto LB agar plates. Plaques were blotted onto nitrocellulose membranes. Membranes were rinsed in Tris-buffered saline with 0.01% Tween-20 (TBST) 3 times and subsequently in blocking solution (20% (v/v) fetal serum in TBST). After washing the membranes in TBST, the first antibody (rabbit anti equistatin IgG) in TBST was added and membranes were treated in solution overnight at 4°C. After that, membranes were washed three times with TBST, and treated with second antibody (goat anti rabbit IgG-horse raddish peroxidase). After final washing in TBST, a visualization with diaminobenzidine as a substrate was performed. Three positive clones were isolated from agar plates and after re-plating, phages were eluted from the surface of the agar plates with TE buffer (5 ml per plate). Phage DNA was isolated using Wizard Lambda Preps DNA isolation kit (Promega) according to the manufacturer's procedure. After restriction analysis with 2 μl of *Eco*RI restriction enzyme (10U/μl) per 3 μg of λDNA and size fractionation on 1% agarose gel, cDNA inserts were excised, purified with glass milk and subcloned into *Eco*RI cloning site of pUC19 plasmid. Whole ligation mixture (10 μl of each sample) was transformed into DH5α *E. coli* cells by incubating the 100 μl of highly competent cells (O.D.<sub>550</sub>=0.6) and 10 μl of ligation mixture in a water bath (42°C) for 45 seconds. After addition of LB medium (900 μl) and 1h incubation (37°C, 250 rpm), bacterial mixture was plated onto LBA plates, supplemented with X-gal and IPTG and after overnight incubation (37°C), white colonies were transferred into 5 ml of LB medium and incubated for an additional 16 hours (37°C, 250 rpm). Plasmids were isolated using Wizard Plasmid Purification System (Promega) according to manufacturer's instructions and analysed by nucleotide sequencing using T7 DNA polymerase (T7 sequencing kit, Pharmacia) and [<sup>35</sup>S]dATPαS (Amersham). Sequencing of selected cDNA

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clone clones resulted in the full length cDNA clone given in Figure 1. The DNA sequence of FIG. 1 corresponds to SEQ ID NO:1. The protein sequence of FIG. 1 corresponds to SEQ ID NO: 2.

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